MICROBIAL POPULATIONS IN A JET-FUEL-CONTAMINATED SHALLOW AQUIFER AT TUSTIN, CALIFORNIA

By Garry G. Ehrlich, Roy A. Schroeder, and Peter Martin

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DONALD PAUL HODEL, Secretary

GEOLOGICAL SURVEY

Dallas L. Peck, Director

For additional information write to:

District Chief
U.S. Geological Survey
Federal Building, Room W-2234
2800 Cottage Way
Sacramento, CA 95825

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CONVERSION FACTORS

For readers who prefer to use metric units rather than inch-pound units, the conversion factors for the terms used in this report are listed below.

Multiply	By	To obtain
in. (inches)	25.40	millimeters
ft (feet)	0.3048	meters
mi (miles)	1.609	kilometers
ft^2 (square feet)	0.09294	square meters

Chemical concentration is given in milligrams per liter (mg/L). Milligrams per liter is a unit expressing the solute per unit volume (liter) of water. For concentrations less than 7,000 mg/L, the numerical value is about the same as for concentrations in parts per million.

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ABSTRACT

JP-5 jet fuel has contaminated 100,000 square feet of the shallow perched aquifer at the U.S. Marine Corps Air Station (Helicopter) in Tustin, California. A 5-foot clay layer provides an impermeable barrier to vertical migration to the deeper main water-bearing aquifer. Horizontal transport results in slow seepage from the banks of the Peters Canyon channel which intercepts the shallow aquifer downgradient from the source of contamination.

Determinations of bacterial populations in the ground water indicate that bacterial counts are 100 to 1,000 times higher inside than outside the zone of contamination. Growth on JP-5-inoculated agar plates shows that substantial populations of JP-5-assimilating bacteria are present in the shallow aquifer. It appears that only a few species of specialized bacteria, presumably those able to assimilate JP-5, are preferentially selected for in the contaminated zone.

Laboratory enrichment experiments show that the addition of nitrate plus phosphate stimulates growth and converts a mixture of fuel and water to an emulsion. The presence of carboxylate metabolites in the ground water suggests that biotransformation of jet-fuel hydrocarbons stops short of complete mineralization to inorganic carbon, presumably reflecting oxygen limitation.

It is proposed that injection of inorganic nitrogen, phosphorus, and oxygen might enhance in-situ cleanup of the aquifer.

Page 3 follows

INTRODUCTION

Jet fuel has contaminated the shallow aquifer at the MCAS(H) [Marine Corps Air Station (Helicopter)] in Tustin, Calif. (fig. 1). Contamination is a result of leakage from unlined earthen pits used for burning waste fuel during crash-crew training exercises. Aerial photographs show that the pits have been present for at least 15 years. The periodic presence of an iridescent oil sheen in Peters Canyon channel (fig. 2), a result of overland flow and seepage from the channel bank, led to detection of the problem in January 1983. The U.S. Geological Survey, at the request of the Marine Corps, has begun a study to determine the areal extent of contamination, direction of contaminant migration, and possible methods for cleanup.

Effective cleanup of aquifers that have been contaminated with petroleum products is often accomplished by depression pumping (de Postrovich and others, 1979). This involves creating a cone of depression in the water table near or in the contaminated zone and then removing the floating hydrocarbon phase that collects in the water columns of wells in the cone by pumping it to the surface. The shallow aquifer at the MCAS(H) consists predominantly of low-permeability materials; hence conventional depression-pumping methods alone might be relatively ineffective for removing the JP-5 fuel from the aquifer.

An alternative scheme under consideration involves coupling biochemical activity with depression pumping. Naturally occurring soil micro-organisms are known to mineralize some components of fuels (Jamison and others, 1973). Some of the micro-organisms also produce hydrocarbon emulsifiers (Gutnick and Rosenberg, 1977). These emulsifying agents could promote dispersion of pockets of trapped hydrocarbons from the sediments. The rate of contaminant removal by depression pumping would be accelerated if dispersion and desorption effects were intensified.

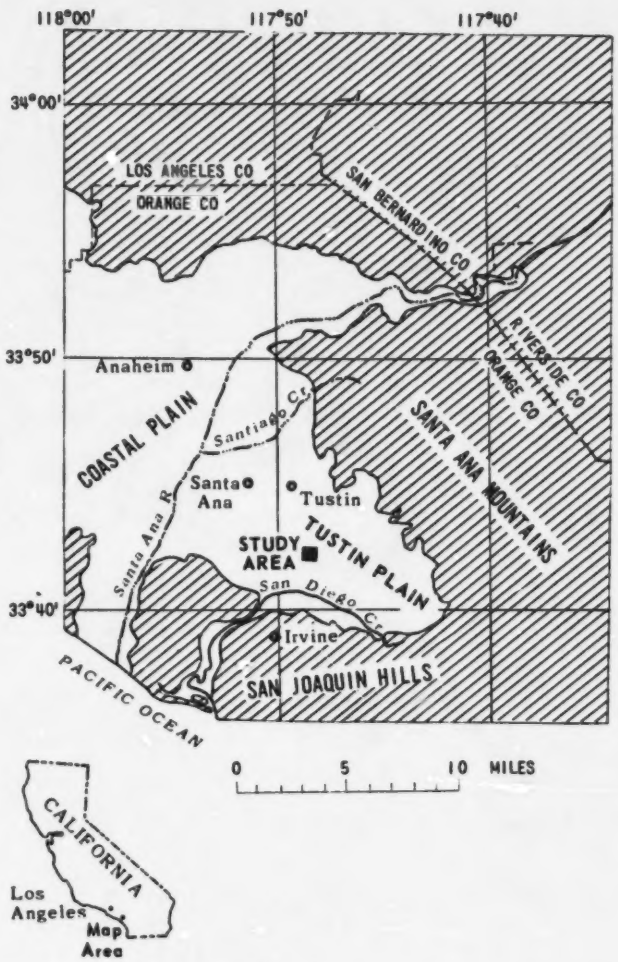


FIGURE 1.—Location of the study area.

This report presents results of investigations on the indigenous hydrocarbon-degrading bacteria at the MCAS(H) site as a background for further development of a comprehensive remedial plan.

Description of the Study Area

The study area (fig. 1) is about 40 miles southeast of Los Angeles in the Tustin Plain. The Tustin Plain lies within the central block of the coastal plain and is bounded on the east by the Santa Ana Mountains and on the south by the San Joaquin Hills. Sediment from these highlands has created the Tustin Plain and makes up the underlying aquifer.

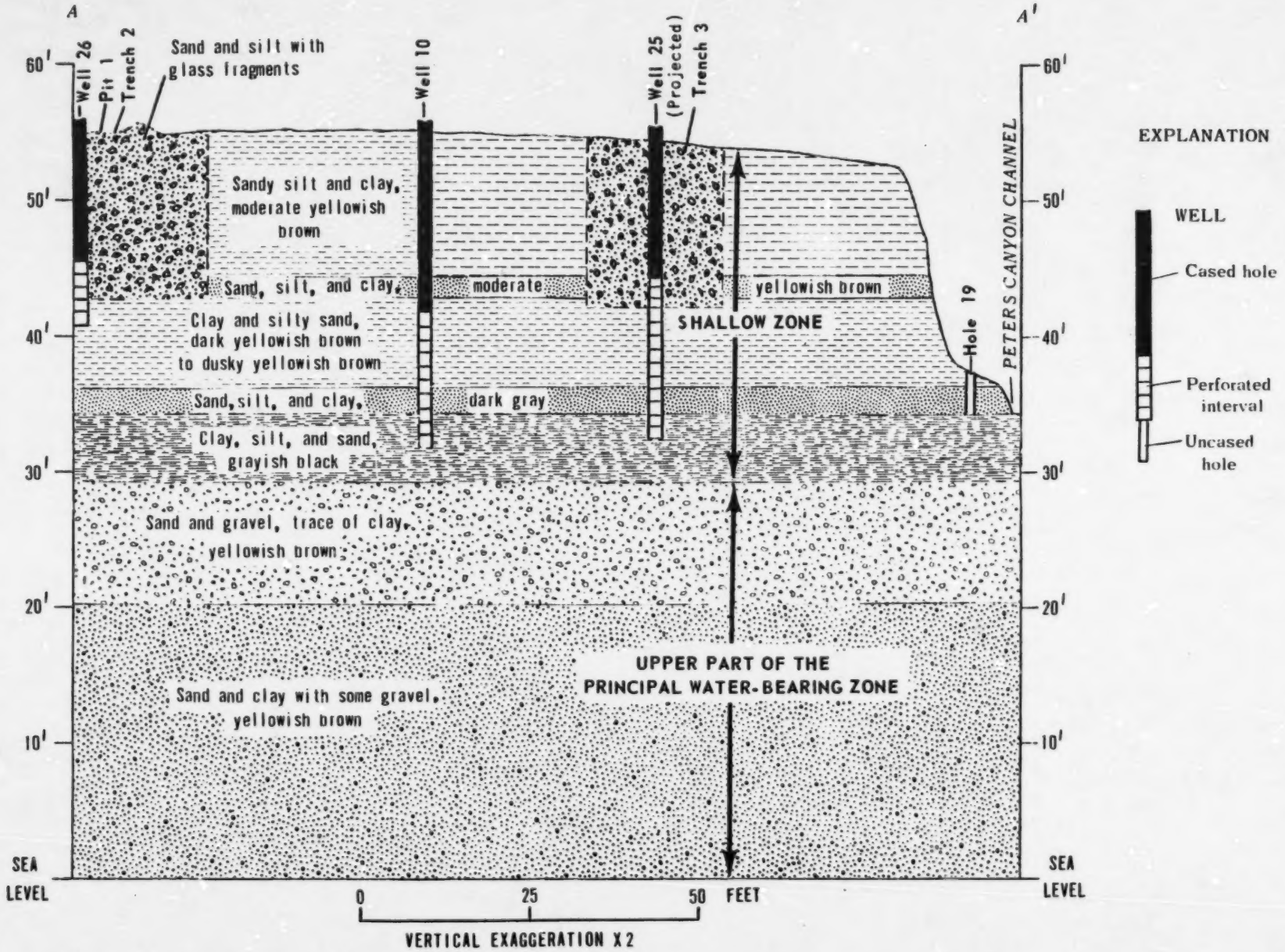


FIGURE 2.—Geologic section in the study area at Tustin, California. Location is shown in figure 3.

Description of the Aquifer System

The aquifer in the Tustin Plain ranges in thickness from 0 feet along the margins of the plain to more than 1,300 feet in the central part. In the study area the aquifer includes a semiperched shallow zone, extending from land surface to a depth of about 26 feet; and the principal water-bearing zone, which extends from about 26 feet below land surface to a depth of about 1,000 feet. The shallow zone consists predominantly of silt and clay interspersed with two thin sand lenses at about 10 and 20 feet below land surface (fig. 2). The bottom of the shallow zone consists of a 5-foot clay layer of low permeability. This zone is unconfined and is recharged by rainfall and irrigation-return flows.

The principal water-bearing zone consists of unconsolidated deposits of Holocene alluvium and partly consolidated deposits of late Pleistocene age. Drillers' logs in the study area indicate that the upper 100 feet of the principal water-bearing zone consists predominantly of silt and clay layers (Hardt and Cordes, 1971, p. 30; Singer, 1973, p. 34). Wells drilled into the alluvium derive most of their water from coarser basal deposits of the alluvium. The Pleistocene deposits include a thick succession of silt- and clay-rich sediments with occasional sand and gravel lenses (Singer, 1973, p. 12). Ground water in the principal water-bearing zone is confined throughout most of the Tustin Plain.

Ground-Water Movement

The spring 1984 water-level contours constructed from measurements in wells perforated in the shallow zone are shown in figure 3. From the burn pits, ground water moves generally southeastward and discharges into Peters Canyon channel. West of the burn pits there is a ground-water divide; west of the divide ground water moves to the west. Ground-water movement is complicated, however, by three filled trenches that parallel the channel (fig. 3). The trenches are about 10 feet deep and are backfilled with material that is more permeable than the surrounding native soil. The differences in permeability cause the water-level contours to deflect in the vicinity of the trenches.

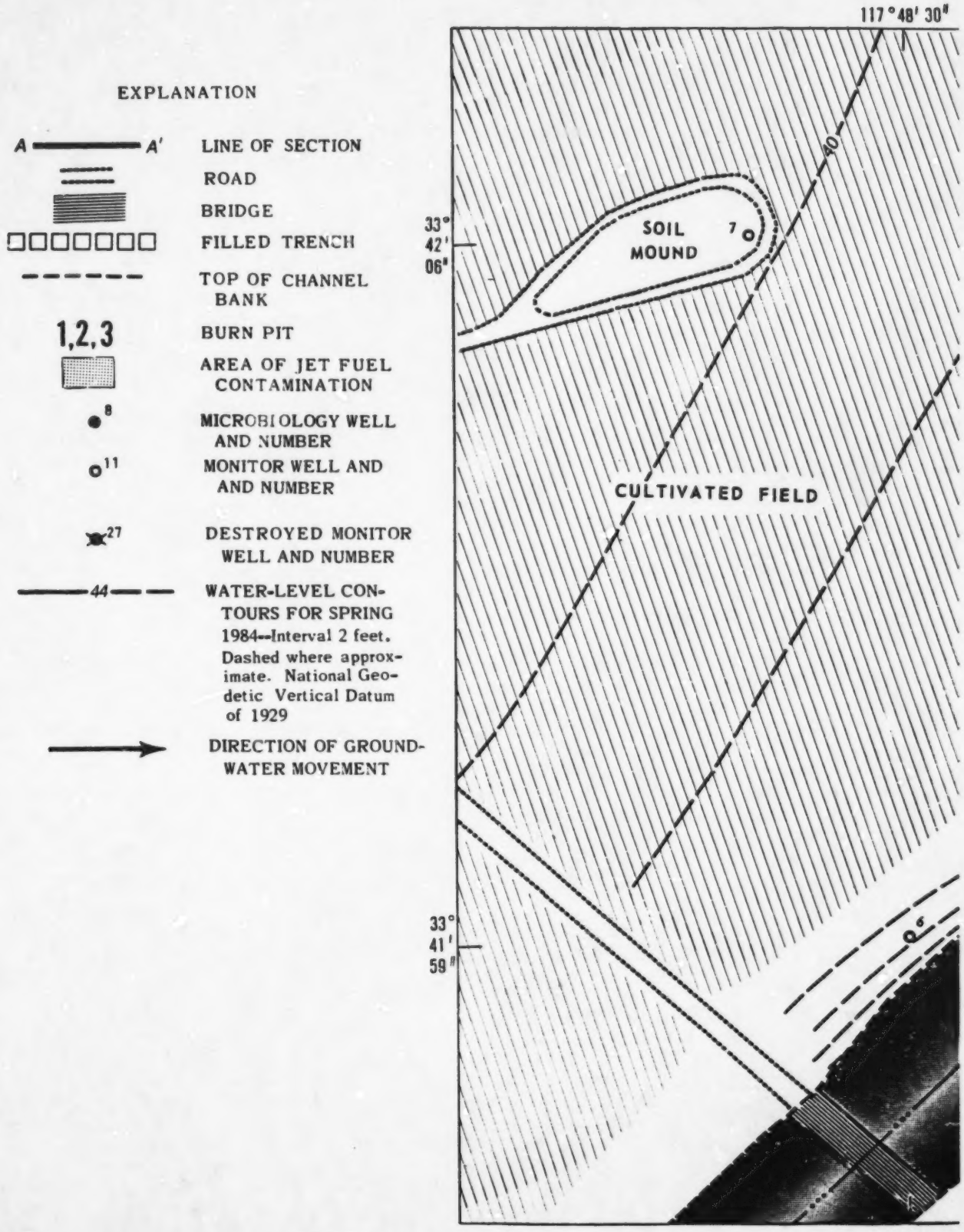
Ground-water movement in the principal water-bearing zone in the vicinity of the study area is generally to the west during the winter months when ground-water pumping is at a minimum. During the summer months, ground-water pumping east of the study area causes a reversal in the ground-water gradient.

Prior to significant ground-water development in the Tustin Plain, ground water moved upward from the principal water-bearing zone to the shallow zone. Ground-water pumping since 1945, however, has lowered the water level in the main water-bearing zone below the water table of the shallow zone, causing water to move downward from the shallow zone to recharge the principal water-bearing zone (Hardt and Cordes, 1971, p. 8). During the study period, water-level fluctuations related to pumping in the principal water-bearing zone were not observed in the shallow zone, indicating that in the study area little hydraulic continuity currently exists between the shallow zone and the underlying heavily pumped aquifer.

MATERIALS AND METHODS

Well Construction

Wells were constructed of 2-inch outside-diameter polyvinyl-chloride casing which was slotted by horizontal saw cuts over the interval from about 9 feet to 21 feet below land surface. The annulus was backfilled with quartz sand from the bottom of the hole to within 3 to 5 feet below land surface and then with cement to land surface. Location of wells is shown in figure 3.


EXPLANATION
A
A'
LINE OF SECTION
ROAD
BRIDGE
FILLED TRENCH
TOP OF CHANNEL BANK
1,2,3
BURN PIT
AREA OF JET FUEL CONTAMINATION
8
MICROBIOLOGY WELL AND NUMBER
11
MONITOR WELL AND AND NUMBER
27
DESTROYED MONITOR WELL AND NUMBER
44
WATER-LEVEL CONTOURS FOR SPRING 1984--Interval 2 feet. Dashed where approximate. National Geodetic Vertical Datum of 1929
DIRECTION OF GROUNDWATER MOVEMENT
117°48' 30"
33° 42' 06"
33° 41' 59"
SOIL MOUND
7
40
CULTIVATED FIELD
6

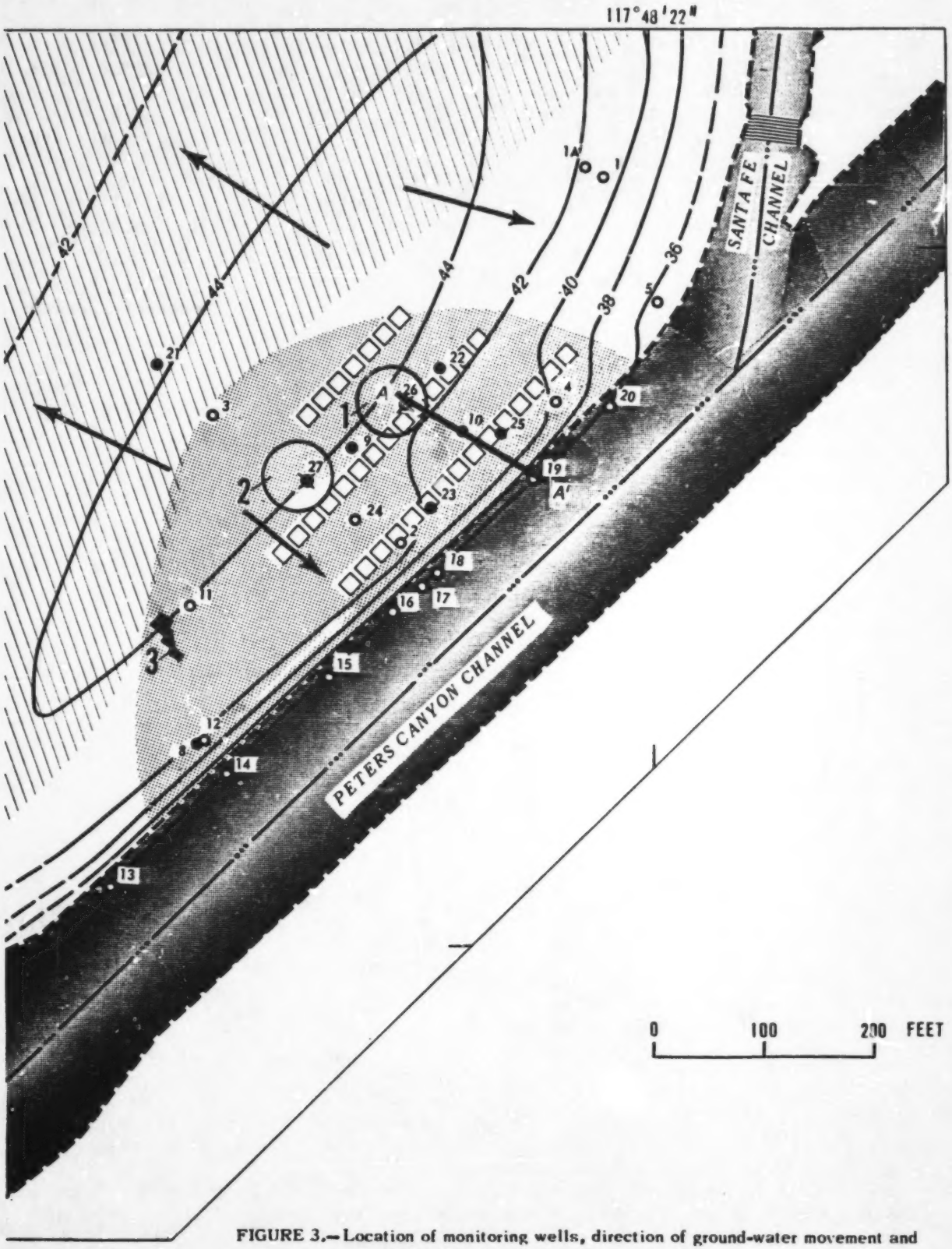


FIGURE 3.—Location of monitoring wells, direction of ground-water movement and estimated area of jet fuel contamination.

Sampling

Samples for microbial analyses were collected from wells 8, 9, 21, 22, 23, and 25 on November 16-17, 1983. Samples from wells 21, 22, and 23 were collected daily from January 16-20, 1984. Samples for chemical analyses were collected in the spring and summer of 1983. Approximately 24 hours prior to sampling, each well was bailed until three casing volumes of water were removed.

Water samples were collected using a glass bailer equipped with a glass ball-check valve at the bottom. Bailers were rinsed with acetone and sterilized in 0.06-percent sodium hypochlorite bleach solution immediately before use. The bailers were lowered into the water until the top of the bailer was just submerged, and then rapidly raised. Samples for enumerating anaerobic microorganisms were immediately drawn into a sterile syringe through a 6-inch-long 16-gauge steel cannula. The cannula was replaced with a hypodermic needle, and test media were inoculated in the field. The remaining water in the bailer was poured into a sterile bottle which was capped and placed in an ice chest for transport to the laboratory for other determinations. All microbiologic testing was completed within 2 hours after collection.

Microbiological Methods

AODC (acridene orange direct counts) were done by the method described by Hobbie and others (1977).

Colony counts were made using spread plates following the guidelines given by Koch (1981). The following media were used: SMA (standard methods agar) (BBL Microbiology Systems, Cockeysville, Md.)[1] and mineral salts agar (Stanier and others, 1966). For determining counts of JP-5-utilizing bacteria, 0.1 mL (milliliter) of well water, or a dilution thereof, was spread over the surface of a sterile plate of mineral salts agar which was then inverted over a piece of JP-5-saturated filter paper contained in the petri-dish lid. Standard methods agar plates were incubated at 30°C for 48 hours. JP-5 plates were incubated at ambient conditions (18-22°C) for 7 days.

Methanogenic bacteria were determined by multiple-tube procedures according to the method of Godsy (1980).

Sulfate-reducing bacteria were determined by multiple-tube procedures using API (American Petroleum Institute) broth (Difco, Detroit, Mich.)[1].

Heterothrophic anaerobic bacteria were determined by multiple-tube techniques using prereduced, anaerobically sterilized, peptone-yeast extract glucose broth (Holdeman and Moore, 1972).

Chemical Analyses

Core-sediment and water samples were analyzed for refined petroleum liquids by the U.S. Geological Survey National Water Quality Laboratory in Arvada, Colo. Hexane extracts were concentrated and analyzed by fused-silica capillary-column gas chromatography with flame-ionization detection. A standard was prepared by dissolving free-floating fuel from one of the wells in hexane. Quantitation was obtained by adding the area of the peaks in the sample extract and then dividing this sum by the summed area of matching peaks in the standard. Retention times had to be within plus or minus 0.02 minute to qualify as a match. On the average, about 50 peaks fulfilled this requirement. Spikes with the jet-fuel standard and C_{20}-hydrocarbon indicated that extraction efficiencies were about 80 percent.

Dissolved methane was determined by the procedure described by the American Public Health Association and others (1976).

[1]Use of corporation and brand names is for identification purposes only and does not imply endorsement by the U.S. Geological Survey.

Fatty-acid analyses were done on a Finnegan 4500 fused-silica capillary-column gas chromatograph-mass spectrometer data system. The pH of 5-mL portions of well water or cell-free laboratory culture fluid was adjusted to 12 with 7N KOH solution and extracted with 5 mL of dichloromethane. The phases were separated and the aqueous phase was acidified with 10N H_2SO_4 to a pH less than 2. Two milliliters of the acidified aqueous phase were extracted with 1.0 mL of diethyl ether. Diethyl ether extracts were dried over sodium sulfate before analysis.

RESULTS AND DISCUSSION

Zone of Contamination

The approximate boundary of contamination is shown by the shaded area in figure 3. The boundary is based on concentration of JP-5 fuel in samples from 20 monitor wells and 8 shallow hand-augered holes. The contaminated area includes about 100,000 ft^2 between the burn pits and Peters Canyon channel.

Results of semiquantitative analyses for JP-5-fuel content of soil cores collected during the construction of the monitor wells indicate that the contamination extends from about 7 feet below land surface to the top of the 5-foot clay layer at about 21 feet below land surface. JP-5 fuel was not found in the clay layer, indicating that the clay layer is an effective barrier to the vertical movement of contamination.

Significant concentrations of JP-5 fuel were identified in soil-core samples collected below the present-day water table. The presence of the jet fuel below the water table suggests that the water level has been lower in the past. Water levels have declined from about 7 feet below land surface in spring 1983 to about 13 feet below land surface in spring 1984. Even larger water-level fluctuations may have occurred in the past, allowing JP-5 fuel to contaminate the entire vertical profile of the shallow zone. Because of the low permeability of the shallow zone, some JP-5 fuel has remained trapped below the present water table.

In the wells sampled for microbiological analyses, thin free-floating fuel layers and dispersed fuel droplets were present in wells 9, 22, 23, and 25. There was no free fuel layer in well 8 but the JP-5 concentration of well 8 water was 19 mg/L (milligrams per liter), which is approximately equal to the solubility in water. Chemical analysis detected no JP-5 fuel in well 21 water.

Nutrient Concentrations

Phosphorus, nitrogen, and oxygen concentrations were determined in water samples from most of the monitor wells in the study area. These macronutrients were measured to establish whether they were affected by in-situ microbial degradation of the jet fuel.

The concentration of dissolved phosphate (PO_4) was generally a few tenths of a milligram per liter, but was highly variable. Variations show no apparent relation to the jet-fuel contamination. Because phosphate has a strong affinity for particle surfaces, sorption on aquifer soils is probably the major control on ground-water concentrations.

The concentration of nitrate-nitrogen (NO_3-N) ranged from 50 mg/L in samples from well 21 to less than 0.1 mg/L (the detection limit) in samples from wells containing jet fuel. Well 21 was completed surrounded by a cultivated field in 1983 and 1984; therefore, the high nitrate is presumed to reflect the use of fertilizer. Nitrate-N ranged from 1 to 5 mg/L in samples from wells outside the cultivated field and outside the area of jet-fuel contamination. Evidently nitrate is completely consumed by microbial populations degrading the jet fuel.

In surface-water sediments, ammonium increases normally accompany nitrate decreases as the sediment become more

anaerobic. However, ammonium-nitrogen (NH_4-N) concentrations were less than 0.1 mg/L throughout the study area--except in the filled trenches, where concentrations ranged from 1 to 2 mg/L. The ammonium ion is strongly sorbed to particle surfaces and exchangeable clays, so higher concentrations within the trenches might be caused by less sorption on the comparatively larger particle-size (less surface area) material with which the trench is filled (see fig. 2).

Oxygen concentration was 3 to 5 mg/L in samples from wells outside the area of jet-fuel contamination but was zero in wells containing jet fuel. The absence of oxygen was accompanied by a strong odor of hydrogen sulfide in wells located in the trenches, and sometimes by a faint odor in contaminated wells outside the trenches. Evidently aerobic bacteria using jet fuel as an energy source rapidly consume all the available oxygen.

The absence of nitrate and oxygen in the ground water contaminated by jet fuel suggests that in-situ jet-fuel degradation might be enhanced if additional oxygen and inorganic nitrogen were available.

Microbiological-Test Results

Results of microbiological determinations are given in tables 1 and 2. It can be seen that AODC values and SMA and JP-5 agar plate counts were highest in the wells with free fuel layers. AODC values ranged from nearly $1x10^6$ cells/mL to

TABLE 1.--JP-5-fuel concentrations, acridene orange direct counts, and aerobic plate counts for two sampling sequences at MCAS(H), Tustin, California

[AODC, acridene orange direct counts; <, less than; ±, plus or minus]

Well No.	JP-5 concentration[1] (mg/L)	Microbiological sampling dates	AODC (cells/mL) X10^3	Aerobic plate counts SMA (cfu/mL) X10^3	Aerobic plate counts JP-5 (cfu/mL) X10^3
8	19	Nov. 16-17, 1983	2,200	62	62
9	[2]13,000	Nov. 16-17, 1983	13,000	1,200	1,300
21	<0.2	Nov. 16-17, 1983	1.3	<1	<1
		Jan. 16-20, 1984	19±8	0.35±0.21	0.33±0.18
22	[2]16,000	Nov. 16-17, 1983	6,600	900	1,400
		Jan. 16-20, 1984	5,600±1,200	1,200±600	1,000±700
23	[2]130	Nov. 16-17, 1983	1,000	90	90
		Jan. 16-20, 1984	700±100	110±80	70±10
25	[2]820	Nov. 16-17, 1983	7,600	710	1,300

[1]Sampled spring 1983

[2]*Fuel layer present*

TABLE 2.--Anaerobic bacterial indicators at MCAS(H), Tustin, California

[<, less than; -, analyzed and not detected; NA, not analyzed; +, present; MPN/mL stands for most probable number per milliliter]

Well No.	Date (1983)	Anaerobes (MPN/mL)	Sulfate reducer	Methanogens	Methane concentration (mg/L)
8	Nov. 16	<30	+	-	-
	Nov. 17	150	+	-	+
9	Nov. 16	<30	+	-	+
	Nov. 17	<30	+	-	+
21	Nov. 16	<30	-	-	NA
	Nov. 17	<30	-	-	-
22	Nov. 16	210	+	-	+
	Nov. 17	750	+	-	+
23	Nov. 16	2,400	+	+	6
	Nov. 17	430	+	+	5
25	Nov. 16	150	+	+	1
	Nov. 17	2,100	+	+	1

slightly more than $1x10^7$ cells/mL in this group of wells, but AODC values in uncontaminated well 21 were less than $2x10^4$ cells/mL--roughly 40-fold less than the lowest value for a well with a free fuel layer. The AODC for well 8, which is near the edge of the JP-5-fuel plume, was $2.2x10^6$ cells/mL. This is near the low end of the range for the contaminated zone but more than 100-fold greater than in uncontaminated well 21.

AODC values are greater than the SMA and JP-5 agar plate counts by factors of about 10 or less for wells 9, 22, 23, and 25, which all have free fuel layers, but are significantly larger than the counts for uncontaminated well 21. The results of the JP-5 agar plate counts indicate that substantial populations of JP-5-fuel-assimilating bacteria exist in the shallow zone. Interestingly, the counts on JP-5 agar and SMA were quite close even though the compositions of these two media differ greatly. SMA is a mixture of polypeptides, glucose, and a few inorganic salts, whereas the only energy sources in the JP-5 agar are the hydrocarbons of the fuel. The significance of this is unknown.

Methane-producing bacteria and dissolved methane were detected in water samples from only wells 23 and 25. The presence of methane-producing bacteria in these wells was surprising. It was expected that any methane-producing bacteria that might be present would perish as a result of exposure to oxygen during well recovery following bailing. However, Patel and others (1984) reported that methane-producing bacteria may be less sensitive to oxygen toxicity than is commonly supposed. They aerated stationary-phase cells of three species of methanogenic bacteria in the absence of reducing agents. Exponential death rates were observed, but the times required to destroy

90 percent of the bacterial standing crop ranged from 3.5 to more than 9 hours. The density of methanogenic bacteria in the saturated zone may be sufficiently high that a small number of them survive even after several hours exposure to oxygen.

Carboxylate Metabolites

Results of fatty-acid analyses of samples from wells 21, 22, 23, and 25 are given in table 3. Only formate, acetate, propionate, and benzoate were found. Higher molecular-weight acids and alcohols were not found. Anaerobic bacteria are generally unable to utilize the compounds in crude oil and its refined derivatives as nutrients. Methane-producing bacteria can assimilate only a limited range of compounds; they are capable of autotrophic growth with H_2 and CO_2, and some species can use formate and acetate as growth substrates. Sulfate-reducing bacteria can utilize certain fatty acids including acetate (Widdle and Pfennig, 1981). The presence of fatty acids and benzoate in water from wells 22, 23, and 25 suggests that biotransformations of hydrocarbons is occurring in the shallow zone, but complete oxidation of hydrocarbons to CO_2 and H_2O by microbial metabolism does not occur. This incomplete mineralization presumably reflects an oxygen limitation.

Nutrient Addition

A series of laboratory experiments using shaking flasks was done to determine if adding nitrogen or phosphorous to a sample of water from well 22 would enhance microbial growth. The control test involved a mixture of well 22 water and 1 mL of JP-5 fuel inoculated with a culture of hydrocarbon-degrading bacteria derived from well 22. After 7 days the SMA plate count reached 2.6×10^5 cfu/mL (colony forming units) and then declined to 4.1×10^4 cfu/mL after 14 days. Comparable results were obtained when well 22 water and JP-5-inoculated water were each enriched with potassium phosphate to a level of 100 mg/L as $P\text{-}PO_4$ or with potassium nitrate to a level of 100 mg/L as $N\text{-}NO_3$. When both potassium phosphate and potassium nitrate were added to the same solution, the SMA count reached 4.5×10^8 cfu/mL after 7 days. Furthermore, the fluid in the shaking flask assumed a milky white color presumably caused by emulsification of the hydrocarbon phase. With the addition of either phosphate or nitrate, but not both, the JP-5 remained in the form of isolated globules floating on the surface of the water. Evidently the addition of both is required to enhance microbial growth and cause emulsification of the fuel/water mixture.

TABLE 3.--Concentration of carboxylic acids in ground water at MCAS(H), Tustin, California, and in laboratory test solutions

[Concentrations in mg/L; <, less than]

Well No.	Formate	Acetate	Propionate	Benzoate
21	<0.1	<0.1	<0.1	0.6
22	<.1	.8	<.1	.9
23	.2	.3	<.1	<.1
25	2.4	.9	<.1	1.3
Laboratory test	9.0	36.7	.2	14.0

Results of analyses for fatty acids in an inoculated sample of well 22 water that had been enriched with phosphate plus nitrate, and shaken for 7 days, is shown in table 3. It can be seen that substantial amounts of formate, acetate, and benzoate are present, even in this well-aerated sample. This suggests that total mineralization may occur only slowly in the presence of free hydrocarbon, even under aerobic conditions. This aspect is under further study.

SUMMARY AND SUGGESTION FOR FUTURE WORK

The results presented show that the presence of JP-5 fuel supports a significant population of hydrocarbon-assimilating organisms in the shallow zone at the MCAS(H). The presence of methane-producing bacteria suggests that oxygen limitation may be a control on growth of the populations of aerobic hydrocarbon-degrading bacteria. The sulfate-reducing bacteria and methane-producing bacteria are apparently using fatty acids that result from the biotransformation of aliphatic and aromatic hydrocarbons in the JP-5 fuel. The results of laboratory shaking-flask experiments indicate that microbial growth in the shallow zone is limited by the availability of inorganic nitrogen and phosphorous.

In-situ microbial biodegradation has been considered to be a promising process for removal of contaminants from ground water. Examples of in-situ microbial activity as an aid to ground-water cleanup were published by McKee and others (1972), Jamison and others (1973), and Van Loocke and others (1975). Jamison and others (1973) observed that bacterial counts in well-water samples from a gasoline-contaminated aquifer increased by 10- to 100-fold after injection of ammonium sulfate solution into the aquifer near the wells. They also speculated that additional oxygen would be needed to achieve the maximum benefit of in-situ microbial degradation of fuel.

These preliminary findings suggest that injection of soluble nitrogen and phosphorous compounds into the shallow zone could stimulate microbial growth and increase biotransformation of contaminating JP-5 fuel. Evidence for oxygen limitation as a control on microbial growth in the shallow zone already exists. Injection of hydrogen peroxide might prove beneficial if, as a result of its chemical or microbial breakdown, a supply of oxygen were released to the ground water.

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